REMARKS

This Amendment, filed in reply to the Office Action dated February 3, 2011, is believed to be fully responsive to each point of objection and rejection raised therein. Accordingly, favorable reconsideration on the merits is respectfully requested.

Claims 47-55, 57, 59-60, 63-67, 69, 73 - 79, 83-84, 86-89, 91-92, 97-99, 101-105, 107, 109-114, 118, 120, 122 and 125-131 are all the claims pending in the Application. Claims 48-55, 57, 59-60, 63, 69, 73-79, 83-84, 86-87 and 97-98 are withdrawn from consideration as allegedly being directed to non-elected inventions. Claims 1-46, 56, 58, 61, 62, 68 and 70-72, 80-82, 85, 90, 93-96, 100, 106, 108, 115-117, 119, 121, 123 and 124 are canceled herewith without prejudice or disclaimer.

Claims 47, 48, 52, 64, 69, 87, 92, 99, 101, 102 and 128-131 are amended herewith. Claims 47, 48 and 64 have been amended to reinstate the options of L, support for which can be found throughout the specification as originally filed, and at, for example, page 8, lines 25-26. Claims 47 and 64 are amended herewith to incorporate the proviso that Lig is not XAC when Fl is BODIPY 630/650; support for this amendment can be found throughout the specification as originally filed, and at, for example, page 28, lines 42-44. Claims 52, 87, 92 and 99 are amended herewith to delete the option that Lig is XAC; support for this amendment can be found throughout the specification as originally filed, and at, for example, page 28, lines 42-44.

Claim 69 is amended herewith to correct claim dependency. Claim 99 is amended herewith to delete the definition of Lig previously excluded from the scope of the claims. Claims 101 and 102 are amended herewith to further define a preferred agonist option. Claim 128 is amended herewith to incorporate the omitted term "combination thereof." Claim 129 is amended herewith to incorporate heteroaryl preferences and a preferred position; support for this

amendment can be found throughout the specification as originally filed, and at, for example, page 21, lines 6-10 and page 22. Claims 130 and 131 are amended herewith to insert a period at the ends of the claims.

No new matter is added by way of this amendment. Entry and consideration of this amendment are respectfully requested.

Claim to Priority

The priority claim to a provisional application 60/465,807 filed 04/28/2003 was timely made on International filing and the priority document timely received at WIPO on 04/27/2004, according to the enclosed Form PCT/IB/304.

Information Disclosure Statements

Applicants thank the Examiner for returning signed and initialed copies of the PTO Form SB/08 that accompanied the Information Disclosure Statement submitted September 20, 2010, indicating consideration of the references therein.

The Obviousness Rejection over Boring, Burchard, Buschmann, Jacobsen, Heefner, Sauer and Cherif

On page 6 of the Office Action, Claims 64-67, 88, 89, 91, 92, 95, 96, 99-104, 107, 109-114, 118, 120, 122, 123 and 125-131 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Boring in view of Burchard, Buschmann, Jacobsen, Heefner, Sauer and Cherif.

Applicants disagree with the rejection, and respectfully traverse for the following reasons.

Initially, Applicants respectfully submit that the Examiner has failed to properly consider the features of dependent claims, in particular claims 101 to 131. For example, no reference has been cited to support the obviousness of conjugates of GPCR agonists, as claimed in claims 101-102. Applicants respectfully request substantive examination of these dependent claims.

Turning to the substance of the rejection, Applicants respectfully note that this new rejection is predicated on essentially the same grounds as the previous rejection, using different references for substantially the same disclosure. Applicants also submit that the Rule 132 Declaration, previously submitted, does not appear to have been accorded due weight. The Examiner maintains that it would have been obvious to interchange the teaching of one fluorescent dye in GPCR context for another fluorescent dye in a different context. However, the inventor Declaration of June, 2010, established that different classes of fluorophore, other than that presently claimed, are not readily interchangeable, and their effect on binding and visualisation varies from class to class.

In this regard, Applicants respectfully point out that the Declaration stated that the evidence of the inventors (*i.e.*, Baker *et al.*, 2010, *British Journal of Pharmacology*, vol. 159:772– 86 2010) is incontrovertible that each molecule, at the time of the invention, had to be designed and assessed each time because of unpredictable pharmacology - the very nature of the process meant that no fluorescent ligand design could have been **obvious** at the time of the invention. Only now, with the inventors' and other's contributions in print in the primary literature, one can use robust & potentially reproducible approaches to design the claimed types of molecule.

Of the newly cited references, the Examiner states that Boring, Jacobson and Heefner disclose XAC conjugated to a non BODIPY fluorophore (fluoresceins, NBD) or other compound

(lipids, peptides) and that Sauer, Cherif and Burchard disclose conjugating non GPCR-ligand molecules (nucleotides, biotin) to BODIPY 630/650. However, Applicants respectfully submit those of ordinary skill in the art would not have possessed a reasonable expectation of success in combining these references in the manner asserted in the rejection, in contrast to the Examiner's position.

For example, the Examiner states at page 10 that "[t]he conjugation of XAC to a fluorescent dye … [and] …of a biomolecule to the fluorescent dye BODIPY 630/650 to generate a compound used in biological experiments is [are] well known in the art. One of ordinary skill would have recognised the interchangeability of the fluorescein of Boring for the BODIPY 630/650 of Sauer, Cherif, Burchard and Buschmann." And at page 11: "The conjugated biomolecules ….[and] … fluorescent dyes (fluoresceins, NBD, BODIPY 630/650) are all small organic molecules of a similar size." …. "Boring and Jacobson [3] teach … fluorescent dyes can be conjugated to XAC, …the C8 position that tolerate substitutions and retain A_1-AR binding, … a linker between XAC and a fluorescent dye that tolerate substitutions and retain A_1-AR binding."

Claims 47 and 64, directed to conjugates of a GPCR ligand with the preferred red emitting BODIPY dyes, particularly suited for binding to and visualization of GPCR receptors, whereby the fluorescence is distinct from both cell autofluorescence and, where present, fluorescence from GFP tagged receptors, are amended herewith to incorporate the subject matter of Claim 122, thereby excluding without prejudice from the claims the conjugates of XAC with BODIPY 630/650. This is based on a proviso to exclude this compound, already present in the application as filed, and thus no issue of adequacy of description arises.

Further, Applicants maintain that while the combination of XAC and BODIPY 630/650

is not obvious, the combination of other GPCR ligands with BODIPY 630/650 was even less so; at the time of the invention, those of ordinary skill in the art would not have have possessed sufficient reason to make such a combination, nor possessed any expectation of success in doing so, when the state of the knowledge at the time of the invention was that GPCR ligands were considered non-interchangeable with other non-GPCR ligands, and with other GPCR ligands alike.

With respect to the "reasonable expectation of success" asserted in the rejection, Applicants note that it is important to consider whether the results (of combining the cited teachings) are predictable, and whether the problem addressed was known.

The results of preparing red-shifted BODIPY fluorescent ligands of non-XAC GPCR ligands are not predictable

Applicants respectfully submit that by exclusion of XAC from the claimed compounds, the obviousness rejection in view of the cited references disclosing XAC as a GPCR ligand conjugated to a fluorophore is overcome. Therefore, even if one of ordinary skill in the art were to consider the fluorescein of Boring, Jacobsen or Heefner interchangeable with the BODIPY 630/650 of Sauer, Cherif, Burchard or Buschmann, they would not have arrived at the invention as claimed, in the absence of a further teaching of the interchangeability of XAC with other GPCR ligands. However, as shown in the previously submitted paper (Baker *et al.*), it is recognized in the art that XAC and other GPCR ligands are not interchangeable, when prepared as conjugates with other non-red shifted BODIPY fluorophores. Each ligand is shown to be differently affected when conjugated to other non-red shifted BODIPY fluorophores. Accordingly, it is entirely unexpected that the red-shifted BODIPY fluorophore, when bound to GPCR ligands in general, displays characteristic properties (retention of pharmacology, in

particular agonism, effective visualization) across the class of GPCR ligands claimed. Those of ordinary skill in the art would not have expected or predicted this result. Accordingly, Applicants respectfully submit that the skilled person would have to first appreciate the interchangeability of both ligand moiety and fluorophore to arrive at the presently claimed invention; however, neither the cited art, nor the state of the art at the time of the invention, provides any teaching to this effect - nor any indication that the results of such interchange are predictable. Indeed, Boring in combination with Sauer, Cherif, Burchard and Buschmann, fail to provide any motivation to effect such interchange.

Notwithstanding the above, Applicants respectfully submnit that even without the deletion of XAC, the present claims are not rendered obvious by the cited references.

The results of preparing red-shifted BODIPY fluorescent ligands of GPCR ligands are not predictable

Initially, Applicants respectfully submit that interchanging the fluorescein of Boring for the preferred or exemplified dyes of Cherif and Burchard would not have yielded the claimed invention, and therefore cannot be basis for predictability of results.

Cherif (GB language equivalent US6,562,959) and Burchard give no preference to, or exemplify dyes other than, BODIPY 630/650. Thus, if one of ordinary skill in the art were to take anything from these references, or be provided any expectation of success in combining their teachings, it is with respect to conjugates with dyes other than BODIPY 630/650. Moreover, one of ordinary skill in the art would have been given no expectation of success with respect to interchanging fluorescein for the non-preferred and non-exemplified BODIPY 630/650 of Cherif and Burchard, by the teaching in relation to the preferred or exemplified dyes of Cherif and Burchard. Further still, Cherif teaches a technique for hybridization of DNA based on

complementarity of nucleotides using sets of DNA probes labelled with 3 to 8 dyes of different and non-overlapping absorption and emission maxima. BODIPY 630/650 is used in the traditional competitive antibody binding technique to reveal digoxygenin. No results are given that would incite any expectation of success in one of ordinary skill in the art in arriving at the presently claimed invention. Burchard, previously considered, teaches a similar technique and uses Cy3 and Cy5 dyes in combination to label a DNA sequence. However, as this is simply used as a tool, no details of fluorescence are given. Applicants therefore submit that those of ordinary skill in the art would not have possessed any expectation of success in arriving at the invention as claimed, given the unpredictability in the art. Moreover, as a result of such unpredictability, those of ordinary skill in the art would have found the results of the present invention unexpected, which is further probative of the non-obviousness of the presently claimed invention.

Second, interchanging the fluorescein of Boring with the non-preferred or non-exemplified BODIPY 630/650 dye of Cherif and Burchard, or the exemplified BODIPY 630/650 dye of Sauer or Buschmann, would not incite any expectation of success in those of ordinary skill in the art, given the unpredictability of the state of the art at the time of the invention.

Sauer relates to a means for fluorescence detection of fluorescent conjugates with mononucleotides conducted in aqueous solution, and does not consider interaction of the mononucleotide conjugate with a further substrate, nor conduct fluorescent measurements in autofluorescing media. Moreover, Buschmann teaches assessing changes in fluorescence of fluorophores conjugated to biotin on binding to protein. However, the protein binding is again observed in water, and not in cell material. For example, Buschmann, at page 200, column 2,

teaches… "[f]rom the data shown in Table 2 it is clear that the addition of streptavidin significantly alters the spectroscopic properties of all conjugates investigated."

Such indicates that a valid deduction cannot be made of the changes that will occur with other conjugates. Accordingly, these references, alone or in combination, incite any expectation of success in those of ordinary skill in the art that the BODIPY 630/650 dye would perform in moderating affinity and binding of GPCR ligand conjugates to receptors, present in autofluorescing cell material of the invention. Nor is the invention predictable from these references.

XAC conjugated to fluorophores and other entities

Boring discloses modifying physicochemical or spectroscopic properties of a series of diisocyanate conjugates of XAC. Fluorescent XAC conjugates (5p, 5q, 10g, 10h) with NBD or FITC fluorophores and (10i, 24) with TRITC, as tracers in receptor binding assays are disclosed, none of which are red fluorescent. No reference is made to visualising fluorescently labelled biological molecules and the requirement that such molecules remain fluorescent and capable of visualisation on binding to GPCRs.

Jacobsen discloses spin, fluorescent or ^{19}F nmr labels for potential applications in characterising adenosine receptors. Jacobsen further discloses enhancing potency of adenosine analogues conjugated to lipids (1987, Febs Lett) and novel XAC analogues (1988, Biochem Pharmacol).

Heefner discloses combinatorial screening assays for differentiating samples by their binding patterns. Some of these include fluorescent labels and are analysed by fluorescence polarisation. Labels include bodipy, fluorescein, coumarin, Newport green, sodium green, phen

green, dichlorofluorescein, none of which are red fluorescent. No reference is made to visualising fluorescently labelled biological molecules.

Bodipy 630/650 conjugated to biological molecules

Sauer discloses an oxazine dye MR121, a rhodamine dye JA53, a carbocyanine dye Cy5, and a Bodipy dye, Bodipy-630/650 as tools for 640nm pulsed diode laser excitation to detect and identify individual fluorescently labelled analyte molecules. Of these, Bodipy 630/650 corresponds to claim 47. Sauer exemplifies Bodipy 630/650, Cy5 and MR121 and does not indicate any preference amongst these.

Cherif discloses a broad spectrum of fluorophores in multicolour fluorescent in-situ hybridisation, page 6 line 10 states that "...fluorophores used for labelling may be selected from cyanine (in particular Cy3, Cy5. Cy5.5 and Cy7), rhodamine, fluorescein (in particular FITC), Bodipy (in particular Bodipy 630/650), Texas Red (in particular Texas Red), Oregon Green and Cascade Blue. Of these Bodipy 630/650 is stated in two of three preferences, for use with a combination of 5 other fluorophores. The examples relate solely to FITC, Cy3, TR, Cy5 and biotin/Cy7.

Burchard discloses fluorescent labels as a preferred option for labels including biotin, radioactive isotopes, antigens and a number of other known classes of label, for analysing polynucleotide sequences which hybridise to a probe. Fluorescent labels include fluorescein and derivatives, rhodamine and its derivatives, texas red, cyanine dyes including Cy3, Cy3.5 and Cy5, BODIPY dyes including BODIPY-Fl, TR, TMR, 630/650 and 650/670; alexa dyes and others. In fact, only Cy3 and Cy5 are exemplified, and hence are indicated as selected over BODIPY dyes and in particular BODIPY630/650 and BODIPY 650/670 which correspond to amended claims 47 and 64, and dependent claims 125-128 and (BODIPY 630/650) 129 - 131.

Buschmann discloses spectroscopic characteristics of 13 red-absorbing fluorescent dyes, Alexa 647, ATTO655, ATTO680, Bodipy 630/650, Cy5, Cy5.5, DiD, DY630, DY635, DY640, DY650, DY655 and EvoBlue and the binding of their conjugates with biotin to streptavidin. Buschmann concludes that there are limitations with Bodipy 630/650 which dimerises in aqueous solution, and that "knowledge of the spectroscopic behaviour of the dyes used under various conditions is crucial for the design of any application."

The results of combining the disclosures of the cited references are not predictable

The inventors discovered that Cy5 gives poor results (Baker *et al.*, BJP 2010, p779 col 1, "XAC-Cy5 (15) were at least 10% less potent than the XAC-Bodipy 630/650 derivative" and at column 2, "…altering the fluorophore to Cy5 …. effectively abolished any measurable affinity of the agonists for the A_1 receptor.") Applicants respectfully submit that neither the favourable binding performance of Bodipy 630/650, nor the poor binding performance of Cy5, would have been predictable to those of ordinary skill in the art at the time of the invention, from Sauer, the cited references, or otherwise. Sauer draws no distinction between Cy5 and BODIPY 630/650, however, Baker *et al.* is evidence that fluorophores affect nucleotide binding differently (insignificantly) to the manner in which they affect GPCR ligand binding (significantly). Accordingly, Sauer cannot be considered to a reliable teaching that would have incited any expectation of success in GPCR binding based on a teaching of nucleotide binding. In fact, were one of ordinary skill in the art to first investigate labelling XAC with Cy5, and find abolition of affinity, there is nothing in Sauer, alone or in combination with the other cited documents, which would lead them to investigate Bodipy 630/650 with any expectation of greater success.

Turning to Cherif, the inventors discovered that both Texas Red and Cy5 are an ineffective option for fluorescent labelling ABEA and XAC, shown in Baker to respectively abolish affinity for ABEA, and give insufficient affinity for XAC to be measured. Applicants respectfully submit that neither the favourable binding performance of Bodipy 630/650, nor the poor binding performance of Texas Red or Cy5, were predictable from Cherif alone or in combination with the other cited documents.

The same comments directed to Sauer also apply to Cherif. Cherif selected TR and Cy5, among others, over Bodipy 630/650, and the skilled person would therefore be prompted to first investigate GPCR conjugates with these fluorophores. Again, were one of ordinary skill in the art to first investigate labelling XAC with Cy5 or TR, and find abolition of affinity, there is nothing in Cherif, alone or in combination with the other cited documents, which would prompt them to investigate Bodipy 630/650, with any expectation of greater success. The same comments apply for Burchard as for Cherif.

Moreover, even assuming *arguendo* that one of ordinary skill in the art would have possessed sufficient reason and motivation to attempt to develop on the disclosures of Boring, Jacobsen and Heefner - and attempt to visualise fluorescently bound XAC, Sauer and Cherif give no indication that any one fluorophore might perform better than any another. In fact, Buschmann teaches that the fluorescence varies from one system to another. That is, were one of ordinary skill in the art to look first at Cy5, having observed at least a 10-fold loss in affinity (see Baker et al, BJP 2010, page 779 col 1 and Discussion para 1), he would not have been motivated to look at fluorescence. Even supposing that one of ordinary skill in the art would have, by chance, looked first at Bodipy 630/650, the result which they would have found, that

visualisation was enhanced on binding, and was replicated for other ligands, would be a truly unexpected and advantageous result, not prompted or suggested by the prior art.

The advantageous results of the presently claimed invention

Applicants again refer the Examiner to the previously submitted Declaration, and supporting evidence in Baker *et al.*, supporting the unexpected results of the presently claimed invention. Specifically, the claimed dyes are shown as universal dyes for all GCPR ligands investigated, giving acceptable or outstanding affinity in all cases. Surprisingly, it was not necessary to select a different dye for each ligand. This was unprecedented in the field at the time of the invention, and thus would undoubtedly have been unexpected to those of ordinary skill in the art.

Moreover the claimed conjugates display the surprising and unexpected property of being capable of visualization with clear distinction from background fluorescence, and moreover from unbound dye, whereby unbound dye need not be washed away. This simplifies visualization and extends its useful application, as well as the accuracy of observations which can be made with the claimed conjugates.

Moreover, there is no teaching in the prior art in relation to maintaining agonism of fluorescent GPCR agonists as in Claims 101 and 102. The prior art discloses fluorescent GPCR antagonists that abolish activity at the GPCR on binding. The task of providing a fluorescent GPCR agonist which retains agonism on binding is neither disclosed nor suggested in the prior art, and there is no teaching which would give an expectation of success nor indeed would indicate the advantage of the claimed fluorophores in binding to GPCR agonists.

Further, there is no teaching in the prior art in relation to the binding lifetime of fluorescent GPCR as in Claim 103, enabling unbound fluorophore to be washed away, thereby visualising only bound fluorophore.

There is also no teaching in the prior art in relation to fluorescent ligands that give low background fluorescence as claimed in Claim 118. As previously pointed out to the Examiner, Claims 117 and 118 recite an unexpected feature of the invention - whereby only the GPCR-bound fluorescent ligand is visible, the unbound ligand showing low background fluorescence whereby it is not visible. This has the particular unexpected advantage, recited in the claims, that unbound ligand need not be washed away before visualization.

Accordingly, the results can be considered as highly accurate, as there is less risk that bound ligand has been dissociated during washing; moreover, as described at page 45, lines 16-20 of thre specification, it is possible to measure fluorescence in real time ("With ligands showing low background fluorescence it is not necessary to remove unbound ligand by washing before performing either confocal microscopy or FCS. It is therefore possible to measure fluorescence with time, in both time and concentration dependent manner.")

In the case of BODIPY 630/650, the fluorophore Fl appears to embed in the cell membrane region in manner that the fluorescence is intensified, in contrast to fluorophore remaining in aqueous phase, outside the cell membrane. This is described and illustrated in the figures of the present application and in the publication by the inventors, Baker *et al.*, *British Journal of Pharmacology*, vol. 159:772– 86 (2010)(copy submitted herewith).

This is a property particular to GPCR conjugates with BODIPY 630/650 and analogues, exhibited only on binding to GPCR receptors and not taught or suggested in the cited prior art. Accordingly, those of ordinary skill in the art would not have possessed any expectation of

success in arriving at the presently claimed invention from the disclosures of the cited references, or that the presently claimed dyes could provide this advantage. Nor would they have sought this advantage because the prior art is silent as to the problem of distinguishing fluorescence of bound and unbound fluorescent conjugate, *i.e.*, this problem was not known from the prior art teaching.

Applicants respectfully refer to the response to the previous Final Office Action, in which Applicants noted that in the Figures of the instant application, the cell membrane incorporating receptors is clearly illustrated. The specification, at page 63 lines 25-31, and page 66, lines 7-10, describes the binding taking place over a period of up to 60 minutes incubation, with visualization at 5 and 30 minutes, *i.e.,* during the incubation period, and importantly therefore still in the presence of unbound fluorescent ligand.

Applicants also previously submitted that, furthermore, in Baker *et al.* (2010), Figure 7 shows good visualization of binding for BODIPY 630/650 (A, B, C, D). However, when the Texas Red fluorophore was used, during incubation the entire field was red and visualization was not possible - only after washing away unbound ligand (E and F) was good visualization of binding achieved. The dansyl class of dyes are also capable of giving clear real-time visualization of bound fluorescent ligand if the dansyl component protrudes from the membrane region where it is quenched/bleached in the extracellular aqueous region and is thus not visible. Referring to Figure 8 in Baker *et al.,* this effect is shown by imaging the fluorescent ligand in two media, mimicking the cell membrane phase (MeOH) or the aqueous medium (HBS). In the case of Texas Red (B) these are not distinguishable, however in the case of BODIPY 630/650, visualization is only possible in MeOH. In the case of dansyl, visualization is possible in both phases, but in MeOH it is short-lived.

Applicants also previously submitted that Figure 9B shows further proof of visualization of ligand binding of XAC-X-BODIPY 630/650. Black dye quenches anything fluorescing in the aqueous environment. As there is no different between A and B it is clear that the fluorescent ligand does not fluoresce in the aqueous environment.

In view of the foregoing, Applicants respectfully request withdrawal of the obviousness rejection.

Double Patenting

On page 12 of the Office Action, the provisional obviousness-type double patenting rejection over co-pending Application No. 11/576,035 is maintained.

Because this rejection is merely provisional in nature, and Applicants need not address it at this time, Applicants defer responding and again respectfully request that it be held in abeyance until such time as allowable subject matter is identified.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The U.S. Patent and Trademark Office is hereby directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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